



## Implications of elevated CO<sub>2</sub> on pelagic carbon fluxes in an Arctic mesocosm study – an elemental mass balance approach

J. Czerny<sup>1</sup>, K. G. Schulz<sup>1,6</sup>, T. Boxhammer<sup>1</sup>, R. G. J. Bellerby<sup>2,3,4</sup>, J. Büdenbender<sup>1</sup>, A. Engel<sup>1</sup>, S. A. Krug<sup>1</sup>, A. Ludwig<sup>1</sup>, K. Nachtigall<sup>1</sup>, G. Nondal<sup>4</sup>, B. Niehoff<sup>5</sup>, A. Silyakova<sup>2</sup>, and U. Riebesell<sup>1</sup>

<sup>1</sup>GEOMAR Helmholtz Centre for Ocean Research Kiel, 24105 Kiel, Germany

<sup>2</sup>Bjerknes Centre for Climate Research, 5007 Bergen, Norway

<sup>3</sup>Geophysical Institute, University of Bergen, 5007 Bergen, Norway

<sup>4</sup>Norwegian Institute for Water Research (NIVA), 5006 Bergen, Norway

<sup>5</sup>Alfred Wegener Institute for Polar and Marine Research, 27515 Bremerhaven, Germany

<sup>6</sup>Centre for Coastal Biogeochemistry, Southern Cross University, Lismore, NSW 2480, Australia

Correspondence to: J. Czerny (jczerny@geomar.de)

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**Abstract.** Recent studies on the impacts of ocean acidification on pelagic communities have identified changes in carbon to nutrient dynamics with related shifts in elemental stoichiometry. In principle, mesocosm experiments provide the opportunity of determining temporal dynamics of all relevant carbon and nutrient pools and, thus, calculating elemental budgets. In practice, attempts to budget mesocosm enclosures are often hampered by uncertainties in some of the measured pools and fluxes, in particular due to uncertainties in constraining air–sea gas exchange, particle sinking, and wall growth. In an Arctic mesocosm study on ocean acidification applying KOSMOS (Kiel Off-Shore Mesocosms for future Ocean Simulation), all relevant element pools and fluxes of carbon, nitrogen and phosphorus were measured, using an improved experimental design intended to narrow down the mentioned uncertainties. Water-column concentrations of particulate and dissolved organic and inorganic matter were determined daily. New approaches for quantitative estimates of material sinking to the bottom of the mesocosms and gas exchange in 48 h temporal resolution as well as estimates of wall growth were developed to close the gaps in element budgets. However, losses elements from the budgets into a sum of insufficiently determined pools were detected, and are principally unavoidable in mesocosm investigation. The comparison of variability patterns of all single measured datasets revealed analytic precision to be the main issue in determination of budgets. Uncertainties in dissolved organic

carbon (DOC), nitrogen (DON) and particulate organic phosphorus (POP) were much higher than the summed error in determination of the same elements in all other pools. With estimates provided for all other major elemental pools, mass balance calculations could be used to infer the temporal development of DOC, DON and POP pools.

Future elevated  $p\text{CO}_2$  was found to enhance net autotrophic community carbon uptake in two of the three experimental phases but did not significantly affect particle elemental composition. Enhanced carbon consumption appears to result in accumulation of dissolved organic carbon under nutrient-recycling summer conditions. This carbon overconsumption effect becomes evident from mass balance calculations, but was too small to be resolved by direct measurements of dissolved organic matter. Faster nutrient uptake by comparatively small algae at high CO<sub>2</sub> after nutrient addition resulted in reduced production rates under future ocean CO<sub>2</sub> conditions at the end of the experiment. This CO<sub>2</sub> mediated shift towards smaller phytoplankton and enhanced cycling of dissolved matter restricted the development of larger phytoplankton, thus pushing the system towards a retention type food chain with overall negative effects on export potential.

## 1 Introduction

Increasing atmospheric CO<sub>2</sub> concentration is the major man-made geochemical perturbation characterising the anthropocene (Doney, 2010; Revelle and Suess, 1957). Atmospheric CO<sub>2</sub> partial pressure already increased by 40% since the beginning of industrialisation, while rates of CO<sub>2</sub> emissions are continuing to increase beyond most modelled worst-case scenarios, e.g. SRES (Special Report on Emissions Scenarios) A1FI (Meehl et al., 2007; Friedlingstein et al., 2010). Correlations of increasing CO<sub>2</sub> concentrations to global warming are predictable and documented by current measurements as well as in the geological record (Hansen et al., 2006; Petit et al., 1999). About one third of the currently emitted CO<sub>2</sub> is dissolving in the world oceans, serving as a buffer for global climate change (Sabine et al., 2004). However, most of the absorbed CO<sub>2</sub> is accumulating in the surface ocean, separated from deep water masses by thermal stratification, which will further strengthen under global warming. In surface waters, ocean carbonation (increasing CO<sub>2</sub> concentrations) and consequential ocean acidification (decreasing pH) are affecting marine organisms, thereby modulating ecosystem functioning (Riebesell et al., 2009). A predominant geochemical function of the marine planktonic ecosystem is the formation of organic matter from dissolved CO<sub>2</sub>. Sinking of this organic matter, transporting carbon across barriers of physical mixing into the ocean interior is referred to as the biological carbon pump (Sarmiento and Le Quére, 1996; Volk and Hoffert, 1985). Increasing temperatures affect the ocean's function as a physical CO<sub>2</sub> sink, but acidification and carbonation are likely to impact also future biological carbon sequestration (Riebesell and Tortell, 2011; Gruber, 2011).

The Arctic Ocean plays a key role in sequestration of anthropogenic carbon for several reasons. From the physical perspective, high solubility of CO<sub>2</sub> in cold water leads to a high physical sequestration potential of anthropogenic CO<sub>2</sub> in areas of deep water formation. From the biological perspective, nutrient supply to the surface layer during winter deep mixing can promote pulses of high productivity with a large potential for carbon export via the biological pump (Lutz et al., 2007). Whether there is a net release or sequestration of atmospheric carbon depends on the carbon to nutrient ratio and sinking rate of the material exported after a mixing event. These properties are known to be highly variable, depending on food web structure. Deep sedimentation events in the Arctic Ocean may occur when fast sinking particles are formed, i.e. through coagulation of diatom blooms (Klaas and Archer, 2002) or by pteropods and their sticky nets (Bathmann et al., 1991). But efficient recycling of material by a surface layer retention food web may keep export at a low level (von Bodungen et al., 1995). Ocean warming and acidification are expected to impact ecosystems particularly in Arctic regions. Sea-surface warming will likely result in elevated primary production due to a reduction of

sea ice cover and the shoaling of mixed layer depth in the light limited Arctic. Estimates for these effects of sea-surface warming are implemented in various global carbon flux models (Bopp et al., 2001; Sarmiento et al., 2004; Schmittner et al., 2008), whereas effects of changing seawater carbonate chemistry and pH on Arctic community export production are more difficult to quantify.

Ocean acidification and carbonation have the potential to directly affect ecosystem functioning in numerous ways. Of those, the adverse effect of future decreasing pH and carbonate ion concentrations on marine calcification is the most investigated mechanism (Riebesell, 2004; Fabry et al., 2008). Pteropods and foraminifera, the only calcifying organisms of significant relevance to the high Arctic pelagic ecosystem examined, were found to be very sensitive to ocean acidification (Comeau et al., 2009; Lombard et al., 2010; Lischka et al., 2011). Carbonation is also found to directly affect photoautotrophic carbon fixation of cultured marine phytoplankton and natural plankton assemblages (Riebesell and Tortell, 2011). In most studies, elevated CO<sub>2</sub> is reported to enhance carbon fixation, however, CO<sub>2</sub> affinity differs between different algal taxonomic groups (Reinfelder, 2011). In addition to effects of ocean carbonation on primary production, bacterial enzymatic rates were found to be directly affected by decreasing seawater pH (Piontek et al., 2010). Moreover, chemical speciation of potentially limiting micronutrients seems to be pH dependent in the range of projected ocean acidification (Millero et al., 2009).

Increased inorganic carbon consumption in response to elevated *p*CO<sub>2</sub> was already found in incubated natural plankton communities of the North Atlantic (Hein and Sand-Jensen, 1997) and in mesocosm studies in a Norwegian Fjord compiled by Riebesell et al. (2008). CO<sub>2</sub> induced overconsumption of carbon that was presumably exuded by phytoplankton cells in form of organic matter, which was measured as transparent exopolymer particles (TEP). The sticky carbon-rich TEP aggregated with other particles in the water column, thereby potentially increasing carbon export (Engel, 2002; Engel et al., 2004a). Enrichment of carbon relative to nitrogen in the exported matter would cause a substantial increase in the total amount of carbon sequestered in the future ocean (Oschlies, 2009).

This study investigates the response of a natural Arctic plankton community, including all trophic levels from bacteria to millimetre-sized zooplankton, to changes in seawater carbonate chemistry. Treatment levels ranged from ~180 µatm CO<sub>2</sub>, as prevailing during the early summer situation in the beginning of the experiment, over present day and year 2100 atmospheric projections to extreme values of up to 1420 µatm CO<sub>2</sub>. Using tracer gas exchange measurements and quantitative high resolution sediment sampling, we are able to present daily budget calculations for carbon, nitrogen, and phosphorus in all mesocosms. Mass balance calculations were used to crosscheck measured trends and to provide a quantitative evaluation of biogeochemical

fluxes. A “Pool X” representing the carbon not reproducibly measured on a daily basis will be discussed to evaluate the performance of the applied methods and to identify problematic measurement parameters. Those parameters (DOC, DON and POP) will be then estimated based on mass balance calculations. CO<sub>2</sub>-induced changes in carbon fluxes within the water column, towards export and in exchange with the atmosphere are detected using correlation statistics. The presented dataset focuses on carbon fixation and export, but is however too unspecific to provide detailed insights into ecosystem functioning. Based on the presented mass balance, elemental cycling and export is discussed with reference to publications on the same experiment, putting biogeochemistry in the context of the observed plankton community composition.

## 2 Material and methods

### 2.1 Experimental set-up

Nine KOSMOS (Kiel Off-Shore Mesocosms for future Ocean Simulation) mesocosms were moored in the Kongsfjord, Svalbard (78°56.2' N, 11°53.6' E). The enclosures were cylindrical polyurethane bags 2 m in diameter, 17 m long and reaching ~15 m deep into the water. The bags were supported by a stainless steel and glass fibre flotation frame and weighted at the bottom with steel rings closable with polycarbonate watertight shutters. The bags reaching 2 m above the water surface were open to the atmosphere. A spiked roof was mounted at the top to prevent birds from resting on the structures and introducing nutrients into the system (Fig. 1). The fjord water, enclosed by lowering of the bags on the 31 May 2010 (t-7), had a salinity of about 34. The mesocosms contained a natural plankton community, though larger plankton and nekton were excluded using a net (3 mm mesh size) covering the upper and lower opening of the mesocosms during filling. After simultaneous watertight closure of the systems on t-5, flotation rings (Fig. 1) were released from the bottom shutters to buoy upwards, unfolding funnel-type sediment traps of 2 m height. This upper ring of the funnel was of the same diameter as the bag (2 m), therefore touching it on all sides. The rather tight fit of the funnel was chosen to minimise sediment losses at the sides but resulted in ~4.5 m<sup>3</sup> of water below the funnel, which had only limited exchange with the bulk of enclosed water above the funnel. Depending on water/bag movement, water exchange between mesocosms and this “dead volume” happened on a timescale of days. Sediment lost into this dead volume was observed by divers to be very low (for calculated estimates see Table 1); nonetheless additions of dissolved substances (e.g. manipulations) to the water column had to mix with this dead volume before reliable budgets could be calculated. Pteropods of the species *Limacina helicina*, individually collected in the fjord, were added to each mesocosm (100, 20

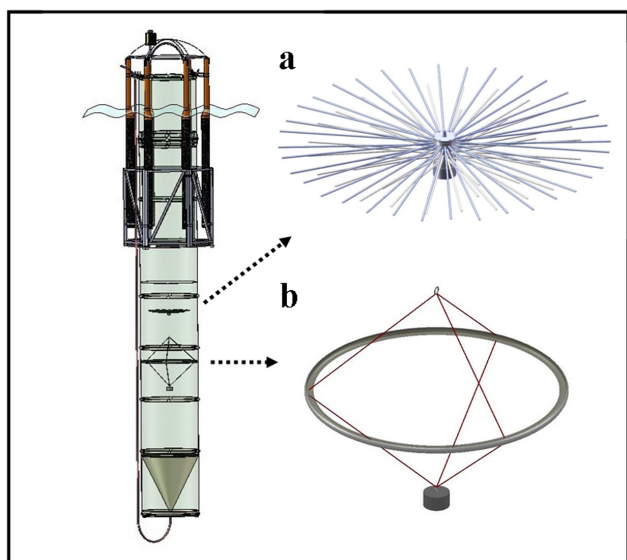
and 70 individuals on day t4, t5 and t6, respectively). Most of them died within a few days in the tight gap between sediment funnel and mesocosm wall. Dead pteropods were picked from sediment samples or ended up in the dead volume. For more detail on the set-up see Riebesell et al. (2013) and find detailed information on abiotic conditions and standing stock succession of phytoplankton, dissolved and particulate matter in Schulz et al. (2013).

### 2.2 Manipulation

The water volume enclosed in flexible wall mesocosms can vary by some percent and was therefore determined using small additions of a calibrated NaCl solution as a tracer (~1 mL L<sup>-1</sup> = 0.2 g L<sup>-1</sup>). Salinity measurements allowed calculation of the enclosed water volume of each individual mesocosm with uncertainties of less than 1 % (Czerny et al., 2013a). Measurements were performed twice, on t-4 and t4, because of considerable uncertainties in salinity measured before the first tracer addition on t-4. Measured volumes were consulted to plan further manipulation steps and to calculate elemental budgets. From t-1 to t4, CO<sub>2</sub> enriched filtered seawater solution was added to achieve eight CO<sub>2</sub> treatments between 185 and 1420 µatm CO<sub>2</sub>, spanning a pH gradient from 8.31 to 7.51 (measured after mixing with the dead volume on t8). The two ~180 µatm CO<sub>2</sub> “control” mesocosms were undersaturated with respect to atmospheric CO<sub>2</sub> at the time the mesocosms were deployed and were blank manipulated using 55 µm filtered fjord water. All mesocosms were enriched with N<sub>2</sub>O as a gas exchange tracer to a final concentration of ~50 nmol kg<sup>-1</sup> on t4, when the second salt addition was performed. Before sampling on t13, a seawater-based mixed nutrient solution, calculated for the volumes of each single mesocosm, was added to establish equal concentrations of NO<sub>3</sub><sup>-</sup> (5 µmol kg<sup>-1</sup>), PO<sub>4</sub><sup>3-</sup> (0.31 µmol kg<sup>-1</sup>) and SiO<sub>3</sub><sup>2-</sup> (2.5 µmol kg<sup>-1</sup>) in all mesocosms. All additions were performed using the “Spider” injection system (Fig. 1a). For a more detailed description of the mesocosm manipulation technique see Riebesell et al. (2013). And for a description of inorganic nutrient dynamics see Schulz et al. (2013).

### 2.3 Sampling

The entire experiment from filling (t-7) to the last sampling of the mesocosms (t31) lasted 39 days. Sampling started at t-3, two days prior to the beginning of the CO<sub>2</sub> manipulation and lasted until t30 for most variables presented here. Daily water sampling for dissolved and particulate matter (representing total inorganic carbon (CT), dissolved inorganic nitrogen (DIN) and phosphorus (DIP), dissolved organic carbon (DOC), nitrogen (DON) and phosphorus (DOP), as well as particulate carbon (PC), organic nitrogen (PON) and phosphorus (POP)) was between 09:00 and 11:00 LT (local time) from boats. Samples were taken using 5 L depth-integrating



**Fig. 1.** Sketch of one of the nine experimental units. Total view of the mesocosm with (a) manipulation device “Spider” and (b) wall brush.

water samplers (IWS, Hydrobios, Kiel, Germany) that delivered one mixed sample from the upper 12 m, representing total water-column inventories. Sampling depth was restricted to 12 m to prevent dispersion of sediment from the 2 m-high collecting funnel at the bottom of the mesocosms. Immediately after water collection, gas samples for N<sub>2</sub>O and carbonate chemistry (CT and total alkalinity (TALK)) were collected directly from the sampler into gas-tight bottles. The rest of the water was transferred into 20 L polyethylene carboys using a silicon tube. Carboys were transported into the lab, screening them from sunlight using black plastic foil. Samples for dissolved and particulate matter were subsampled from carboys. Vertical (0–12 m depth) Apstein net hauls (opening Ø 17 cm, mesh size 55 µm) were performed to determine zooplankton abundance and biomass. To minimise effects on zooplankton density, samples were taken only once per week (days t-2, t<sub>2</sub>, t<sub>11</sub>, t<sub>18</sub>, t<sub>24</sub> and t<sub>30</sub>). For more details see Niehoff et al. (2013).

Sediment was sampled every other day using silicone tubes (10 mm inner diameter) connecting the tips of the sediment funnels to the water surface (Fig. 1). Sediment dispersed in approximately 3 L of water was drawn from the silicone tube into a glass bottle under low vacuum pressure. After gravimetric determination of the sediment-water volume sampled, 20 to 30 mL sub-samples for microscopic inspection were taken with a pipette from the stirred bottles. For bulk chemical analyses (PC, PON, POP and biogenic silica (BSi)), particles were concentrated by settling and centrifugation, and the resulting compact sediment pellets were frozen at –80 °C.

## 2.4 Measurement procedures

Aliquots of water samples for analyses of chlorophyll *a* (Chl *a*; 250–500 mL), PC, PON and POP (400–500 mL) were filtered under low vacuum (200 mbar) on glass fibre filters (Whatman GF/F 25 mm Ø, pre-combusted at 450 °C for 5 h) and stored frozen at –20 °C. Chl *a* was determined fluorometrically according to Welschmeyer (1994) using a Turner fluorometer 10-AU (Turner BioSystems). Quantification of PC and PON was carried out using an elemental analyser (EuroVektor EA) according to Sharp (1974). POP and BSi was determined following the method by Hansen and Koroleff in Grasshoff et al. (1983). For POP this method was modified to the measurement of samples on glass fibre filters. Here, particulate matter was completely oxidised by heating the filters in 50 mL glass bottles with 40 mL of purified water and the reagent Oxisolv (Merck) in a pressure cooker. Solutions were measured colorimetrically on a Hitachi U 2000 spectrophotometer. DOC, DON and DOP were determined from filtered (Whatman GF/F 25 mm Ø, pre-combusted at 450 °C for 5 h) water samples. DOC was analysed on a Shimadzu TOC<sub>V</sub>CN using the HTCO (high-temperature catalytic oxidation) method (Qian and Mopper, 1996); for details see Engel et al. (2013). DON and DOP were oxidised as described for POP and subsequently measured as inorganic nutrients, for nitrate (NO<sub>3</sub><sup>–</sup>), nitrite (NO<sub>2</sub><sup>–</sup>) and phosphate (PO<sub>4</sub><sup>3–</sup>) according to Hansen and Koroleff (1999), and for ammonia (NH<sub>4</sub><sup>+</sup>) according to Holmes et al. (1999). The sum of NO<sub>3</sub><sup>–</sup>, NO<sub>2</sub><sup>–</sup> and NH<sub>4</sub><sup>+</sup> is presented as dissolved inorganic nitrogen (DIN). For a more detailed description of particulate matter (POM) and dissolved organic matter (DOM) and inorganic matter (DIM) analyses see Schulz et al. (2013). CT was determined via coulometric titration using a SOMMA (Single Operator Multiparameter Metabolic Analyser) system, and TALK via potentiometric titration (Dickson, 1981). CO<sub>2</sub> concentrations, partial pressures and pH (total scale) were calculated from CT and TALK measurements with the program CO<sub>2</sub>SYN by Lewis and Wallace (1998). For more details on carbonate chemistry see Bellerby et al. (2012).

Frozen sediment pellets were freeze dried at a pressure of ~ 50 mbar for 1–2 days without additional heat input until room temperature was reached. Subsequently, the dried sediment was ground in a stainless steel ball mill at low temperatures using liquid nitrogen to cool the sample before grinding. Sub-samples adjusted to the analytic measurement range (5–10 mg) were weighed on a precision scale. Analyses of particulate matter were performed as described above for PC, PON, POP and BSi on filters. 20 to 30 mL sub-samples of the sediment suspension, as well as samples from vertical net hauls were analysed for zooplankton composition, abundance and biomass under a dissecting microscope. At the same time, sediment samples were also inspected for general composition (detritus and phytoplankton). Zooplankton was sorted into tin cups for bulk carbon and nitrogen analyses as

described above for water-column PC and PON. For more details on zooplankton analyses see Niehoff et al. (2013).

## 2.5 CO<sub>2</sub> gas exchange estimate

Daily air–sea gas exchange velocities ( $k$ ) were estimated using N<sub>2</sub>O as a gas tracer. Calculations were based on fitted concentration decrease inside the mesocosm in relation to calculated equilibrium concentrations estimated using water  $T/S$  and atmospheric N<sub>2</sub>O concentrations monitored on Zeppelin Mountain (~4 km distance from the experimental site). Daily CO<sub>2</sub> fluxes were calculated using  $k$  (adapted to CO<sub>2</sub> using published Schmidt numbers) together with CO<sub>2</sub> air–sea diffusion gradients. CO<sub>2</sub> gradients were derived from bulk aquatic CO<sub>2</sub> concentrations calculated from CT and AT as well as estimated using bulk water  $T/S$  and atmospheric CO<sub>2</sub> concentrations recorded on Zeppelin Mountain. Corrections for chemical enhancement by Hoover and Berkshire (1969) were applied. For a detailed discussion on gas exchange measurements in mesocosms including complete references on used constants see Czerny et al. (2013b). Zeppelin Mountain atmospheric data were provided by Thomas Conway from the NOAA Carbon Cycles Gases Group in Boulder, US, and Johan Ström from ITM (Department of Applied Environmental Science), Stockholm University, Sweden; atmospheric N<sub>2</sub>O and CO<sub>2</sub> measurements, respectively.

## 2.6 Wall growth estimate

After sampling on the last day of the experiment (t30), a special brush (Fig. 1b) was used to mechanically remove and suspend biomass growing on the inner surface of the mesocosm bag as described in Riebesell et al. (2013). The detached biomass was then quantified by POM measurements of the water column before and right after brushing as described above.

## 2.7 Data presentation

Budget calculations for carbon (C), nitrogen (N) and phosphorus (P) are based on changes in pool sizes over time ( $\Delta\text{pool}$ ) relative to a reference point in time. The addition of CO<sub>2</sub> enriched seawater caused major changes in the CT budget that could only be precisely quantified by direct measurements of CT inside the mesocosms. The earliest reference points for CT budgets were measured after complete mixing of the water column above and below the sediment traps. In the high CO<sub>2</sub> mesocosms this took until t8, while CT values were found to be stable in the non-manipulated control and in some low CO<sub>2</sub> mesocosms earlier. N and P budgets were calculated starting on t13, after inorganic nutrients were added. As a reference value for  $\Delta\text{DIN}$  and  $\Delta\text{DIP}$  from t13 onwards, concentrations measured on t12 plus the known amounts of added inorganic nutrients were used. This particular reference point was chosen because, in this way, budget calculation could be started on the day of nutrient ad-

dition, e.g. before the added nutrients were fully mixed into the dead volume below the sediment traps (see Sect. 2.1). For dissolved and particulate organic matter, starting values (t0, t8, t13, t20) for the analysed phases were obtained by averaging measured concentrations of the respective days with those of the days before and after. As measurement precision for inorganic matter is much higher than for organic material, averaging over three consecutive days was not needed and the concentrations of dissolved inorganic pools measured on the reference days were used as starting values.

Measured changes in inorganic matter that cannot be accounted for by the combined changes in pools of dissolved and particulate organic matter, cumulative gas exchange and sedimentation were assigned as “Pool X”, representing a combination of measurement errors and the following pools unaccounted for in daily sampling: (1) a small amount of sedimented material accumulated in the space surrounding the sediment trap (dead volume) that could not be sampled. (2) A biofilm growing on the mesocosm walls became visible towards the end of the experiment. The size of this pool was estimated from water-column measurements taken immediately before and after brushing the mesocosm walls. (3) Fast-swimming zooplankton such as copepods were not quantitatively sampled with the depth-integrating sampler and are therefore not adequately represented in PC/PON/POP measurements. Biomass of zooplankton larger than 55  $\mu\text{m}$  was estimated by weekly Apstein net hauls. Estimates for these three contributors to Pool X within the three phases of the experiment are based on theoretical considerations combined with available data on wall growth, zooplankton, and sediments (Table 1). Due to a rather constant elemental composition of particulate matter close to Redfield ratios of 106/16/1, those uncertainties in nitrogen and phosphorus are proportionally smaller than presented uncertainties in carbon (data not shown).

Corrections for the effect of water evaporation on dissolved and particulate matter concentrations as well as corrections for the effect of sampling-derived volume decrease on the calculation of air–sea and sediment fluxes were not included to keep calculations traceable. With a range of ~0.2 % and ~1 %, respectively, over the whole experiment, corrections are below the detection limit of most of the applied methods. Moreover, the effects of small changes in water volume would be identical in all mesocosms.

Changes in Pool X ( $\Delta X_{C/N/P}$ ) were calculated for the three phases of the experiment as the summed amount of changes in all major pools as:

$$\Delta X_C = \Delta\text{CT} + \Delta\text{GX} + \Delta\text{PC} + \Delta\text{DOC} + \Delta\text{Sed}_C, \quad (1)$$

$$\Delta X_N = \Delta\text{DIN} + \Delta\text{PON} + \Delta\text{DON} + \Delta\text{Sed}_N, \quad (2)$$

$$\Delta X_P = \Delta\text{DIP} + \Delta\text{POP} + \Delta\text{DOP} + \Delta\text{Sed}_P. \quad (3)$$

**Table 1.** Estimates for the contribution of undetermined carbon pools (or not daily determined pools) to Pool X or  $\Delta\text{DOC}_{\text{calc}}$ . Sediment losses were estimated on the basis of the ratio of areas of funnel opening relative to the gap around the funnel and the average cumulative sediment trapped during each phase. The area from which particles would collect in the gap was estimated as radius  $\times$  length of the sediment trap flotation ring. Copepod biomass changes were estimated from average counts and carbon content determined from weekly net hauls. The depicted value was calculated assuming copepods were not at all represented in the particulate carbon measurements from water samples. Wall growth for phases II and III (after nutrient addition) was estimated, assuming exponential growth of the wall-grown carbon measured on t30 ( $8.31 \pm 3.1 \mu\text{mol kg}^{-1}$ ) at rates of 1 to 0.3 per day (Hagseth et al., 1992). “Maximum contribution of undetermined pools” depicts the sum of estimated mean changes within the three listed pools plus one standard deviation, or in the case of wall growth, the largest estimate.

Phase	I [ $\mu\text{mol kg}^{-1}$ ]	II [ $\mu\text{mol kg}^{-1}$ ]	III [ $\mu\text{mol kg}^{-1}$ ]
Sediment lost into dead volume	$0.046 \pm 0.007$	$0.10 \pm 0.015$	$0.17 \pm 0.063$
Copepod biomass changes	$0.0 \pm 0.11$	$0.26 \pm 0.43$	$1.11 \pm 0.78$
Wall growth	none	0.081 to 0.82	1.0 to 3.4
Maximum contribution of undetermined pools	0.12	1.63	5.52
Observed range of $\Delta\text{DOC}_{\text{calc}}$ on the last day of the phase	−0.93 to 11.09	−7.02 to 8.81	19.32 to 30.5

With  $\Delta\text{CT}$  = change in total inorganic carbon concentrations,  $\Delta\text{GX}$  = cumulative exchange of carbon with the atmosphere (flux in = negative/out = positive),  $\Delta\text{PC}$  = change in total particulate carbon concentrations,  $\Delta\text{DOC}$  = change in dissolved organic carbon concentrations,  $\Delta\text{Sed}$  = cumulative amounts of the respective element found in the sediment trap,  $\Delta\text{DIN}$  = change in dissolved inorganic nitrogen concentrations,  $\Delta\text{PON}$  = change in particulate organic nitrogen concentrations,  $\Delta\text{DON}$  = change in dissolved organic nitrogen concentrations,  $\Delta\text{DIP}$  = change in dissolved inorganic phosphorus concentrations,  $\Delta\text{POP}$  = change in particulate organic phosphorus concentrations,  $\Delta\text{DOP}$  = change in dissolved organic phosphorus concentrations.

Pool X should be ideally zero as changes in one of its components should always be balanced by changes in one or several others. To trace back the source of changes in Pool X, regression analyses were performed, thus testing the degree of variance in all daily Pool X within each phase to be explained by changes in each of the single components on corresponding days (Fig. 2, Table 2). In this way, unexplained variability in measured concentrations of DOC, DON and POP were found to explain most of the variability in Pool X for carbon, nitrogen and phosphorus, respectively. Therefore mass balance calculations were used to estimate possible changes in DOC, DON and POP on the basis of all other budget components. Those estimates are presented as  $\text{DOC}_{\text{calc}}$ ,  $\text{DON}_{\text{calc}}$  and  $\text{POP}_{\text{calc}}$ . Bias in these estimates, composed of summed measurement uncertainties and errors in the combined variables as well as undetermined pools (Table 1), is considerably lower than measurement errors in DOC, DON and POP.

Thus, estimates of  $\text{DOC}_{\text{calc}}$ ,  $\text{DON}_{\text{calc}}$  and  $\text{POP}_{\text{calc}}$  were calculated using mass balances according to the following equations:

$$\Delta\text{DOC}_{\text{calc}} = \Delta\text{CT} + \Delta\text{GX} + \Delta\text{PC} + \Delta\text{Sed}_C, \quad (4)$$

$$\Delta\text{DON}_{\text{calc}} = \Delta\text{DIN} + \Delta\text{PON} + \Delta\text{Sed}_N, \quad (5)$$

$$\Delta\text{POP}_{\text{calc}} = \Delta\text{DIP} + \Delta\text{DOP} + \Delta\text{Sed}_P. \quad (6)$$

As sediment traps were sampled every other day, measured values were linearly interpolated for days in between. Also missing data points of other parameters were linearly interpolated between preceding and subsequent measurements. This applies for DON and DOP of all mesocosms on t16 and t19 and for 8 single data points of DOC.

## 2.8 Statistics

The set-up with 8 different CO<sub>2</sub> treatment levels and no replication of treated mesocosms was designed for regression analyses. Delta values of all tested phases are calculated using the first day of the respective phase as a reference point. Means of changes in measurement parameters ( $\Delta$ values) of single mesocosms during the three phases of the experiment were plotted against mean  $p\text{CO}_2$  in the mesocosms during that specific phase. The null hypothesis that the overall slope is zero and that there is no linear relationship between treatment  $p\text{CO}_2$  and mean  $\Delta$  values was statistically tested with an  $F$  test. Data satisfied assumptions for normal distribution, as confirmed by a Shapiro–Wilk test. Analyses were performed using the program Statistica 6.0 (StatSoft Inc., Tulsa, USA). The same statistical procedure was applied to regression of Pool X to single budget components.

**Table 2.** Linear regression of daily Pool X of all mesocosms during each phase versus corresponding budget components (see Fig. 2). Coefficients of determination depict the share of variances in daily Pool X explained by variances in the datasets it is based on.

Regression of Pool X versus		$\Delta$ PC	$\Delta$ DOC	$\Delta$ CT	$\Delta$ GX	Sed
Phase I	$r^2$	0.01	0.85	0.01	0.00	0.03
	$p$	0.49	0.00	0.51	0.69	0.27
Phase II	$r^2$	0.00	0.90	0.11	0.09	0.10
	$p$	0.89	0.00	0.01	0.02	0.01
Phase III	$r^2$	0.08	0.51	0.24	0.08	0.22
	$p$	0.03	0.00	0.00	0.03	0.00

### 3 Results

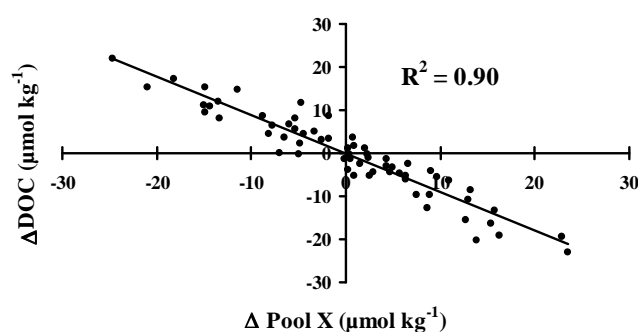
#### 3.1 Chlorophyll *a* and the analysed phases of the experiment

The experiment can be divided into three phases of autotrophic bloom development. An increase in Chl *a* (Fig. 3) started in all mesocosms already during CO<sub>2</sub> manipulation and peaked on t7. A CO<sub>2</sub> effect on Chl *a* concentrations during phase I was not observed. After nutrient addition (t13), there was a lag phase of 2 to 3 days until Chl *a* started to increase. Phase I carbon budget-calculation starts on t8, just after the phase I peak, and is thus describing processes during the bloom decay until t15. During phase II, from t13 onwards, large parts of added inorganic nutrients were consumed and budgets for C, N and P were calculated for build-up and decay of the second bloom peak until t20. Here, the Chl *a* peak of the high CO<sub>2</sub> treatments was higher than the one at intermediate and low CO<sub>2</sub> levels. During phase III, starting on t20, Chl *a* at low and intermediate CO<sub>2</sub> started to increase exponentially, reaching maximum concentrations on t27 and declining thereafter. In contrast, at high  $p$ CO<sub>2</sub> Chl *a* increased more slowly, not reaching peak values until the end of the experiment.

Maximal Chl *a* concentrations of  $\sim 3 \mu\text{g L}^{-1}$  during the course of the experiment are rather low considering the  $5 \mu\text{mol kg}^{-1} \text{NO}_3^-$  and  $0.32 \mu\text{mol kg}^{-1} \text{PO}_4^{3-}$  added. Chl *a* concentrations were transformed into estimates of organic carbon for the autotrophic community using Chl *a* to carbon ratios by Li et al. (2010), with  $0.02 \text{ g Chl } a \text{ g C}^{-1}$  for a phytoplankton community with low contribution of diatoms. Transformation reveals that the contribution of photoautotrophic biomass (up to  $15 \mu\text{mol kg}^{-1} \text{C}$ ) to the standing stock of particulate carbon, PC, (up to  $40 \mu\text{mol kg}^{-1}$ ) was rather low.

#### 3.2 Carbon budget of the replicated control treatment

In Fig. 4 carbon pools of the untreated control mesocosms are plotted over the entire period of the experiment. The bloom development as displayed in Chl *a* (Fig. 3) is mirrored by build-up of  $\Delta$ PC, while cumulative sedimentation of this

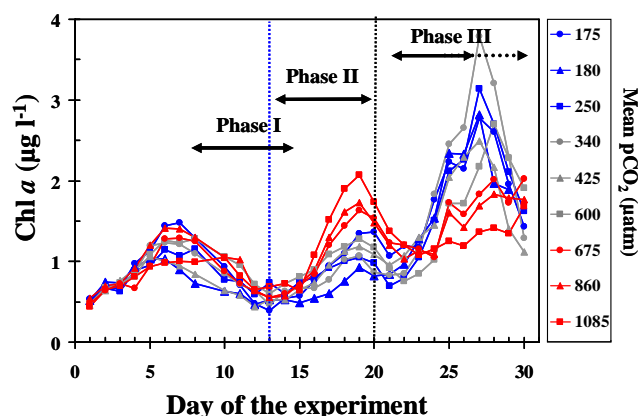


**Fig. 2.** Linear regression of changes in all daily total Pool X ( $\Sigma \Delta$ PC,  $\Delta$ DOC,  $\Delta$ CT,  $\Delta$ Sed,  $\Delta$ GX) estimates within phase II to corresponding changes in measured DOC on the same days can be used to identify measured quantities of DOC to be unrealistic. Regression results of total Pool X to other carbon pools are given within Table 2.

biomass was rather low (Fig. 4a). Until t8, autotrophic uptake of inorganic carbon resulted in a simultaneous decrease of  $\Delta$ CT, which was partly compensated by in-gassing of CO<sub>2</sub> from the atmosphere. Hereafter biomass decreased and PC was partly respired back into the CT pool, sedimenting out or being released as DOC<sub>calc</sub>. Three days after the addition of inorganic nutrients (t16 up to t19),  $\Delta$ PC build up combined with sedimentation was not leading to changes in  $\Delta$ CT but was compensated by CO<sub>2</sub> entering the mesocosms from the atmosphere. During the first 19 days, the sum of particulate carbon produced in the control mesocosms roughly equalled the sum of inorganic carbon consumed, indicated by DOC<sub>calc</sub> approaching zero during this period. Measured DOC values are included in Fig. 4b. Apparently, day-to-day variability in this dataset is much larger than the amount of carbon potentially available for the formation of DOC. This is evident from the sum of other measured carbon species shown as DOC<sub>calc</sub> in Fig. 4a. Parallel variations of Pool X to DOC data (Figs. 2, 4b) identify measurement results to be unrealistic.

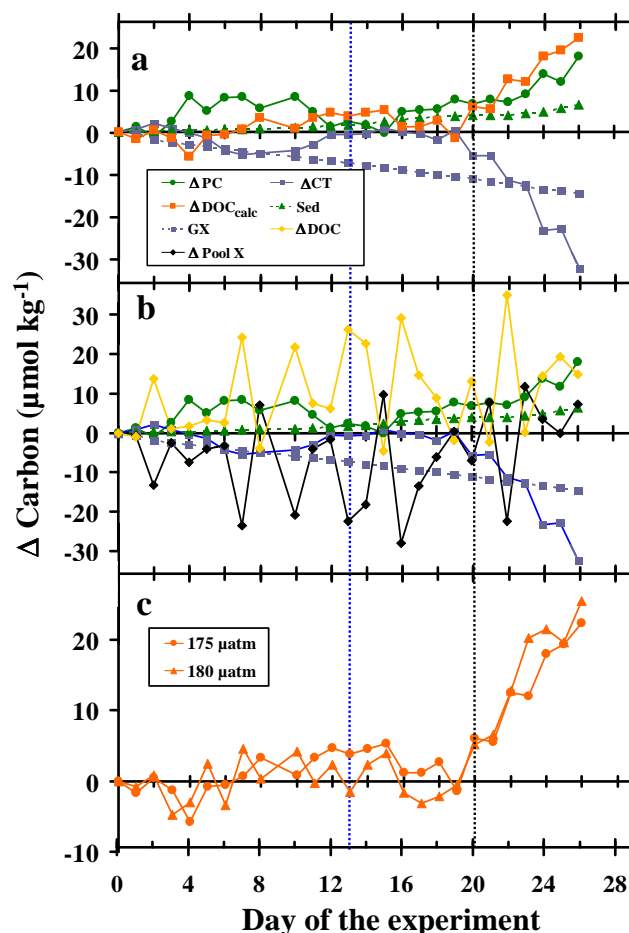
As shown in Fig. 4c, the temporal development of DOC<sub>calc</sub> in the two control treatments was very similar. Variability of DOC<sub>calc</sub> during most of the experimental time was relatively small (DOC<sub>calc</sub> was on average  $-0.43 \pm 2.7 \mu\text{mol kg}^{-1}$  for





**Fig. 3.** Development of chlorophyll *a* concentrations during the course of the experiment. CO<sub>2</sub> partial pressures given in the figure legend are mean values for the experiment (day 8–26). High *p*CO<sub>2</sub> is denoted in red, intermediate in grey and low levels in blue. Within these categories, circles symbolise the lowest, triangles intermediate and squares the highest treatment *p*CO<sub>2</sub> level. The blue dashed line marks the nutrient addition (t13) at the end of phase I. The black dashed line denotes the end of phase II comprising the second bloom peak, phase III corresponds to the third bloom until the end of measurements. Arrows depict time periods used for statistical analyses.

the two controls until t19). Bias of DOC<sub>calc</sub> estimates given in Table 1 could have caused a gradual increase in the DOC<sub>calc</sub> of maximum 1.8 µmol kg<sup>-1</sup> for this period. From t19 to t27, a phytoplankton bloom developed in the mesocosms, extensively consuming inorganic carbon. Increasing ΔPC values and cumulative sedimentation did not balance the sum of 44 µmol kg<sup>-1</sup> CT taken up from the dissolved pool plus the CO<sub>2</sub> entering from the atmosphere (Fig. 4a). It therefore required 19 µmol kg<sup>-1</sup> DOC<sub>calc</sub> to close the carbon budget on t27. Within this period, biomass growing attached to the walls became visible and was estimated to account for 8.3 ± 3.1 µmol kg<sup>-1</sup> of Pool X on t30 averaged over all mesocosms. This biomass was extrapolated back to the two nutrient-replete phases of the experiment based on assumed exponential growth at rates between 0.3 and 1.0, typical for ice algae communities (Hagseth et al., 1992). Even at the rather low growth rates, most of the wall grown biomass developed within the last few days before measurement, thus contributed shares to community biomass were negligible during phase I and II and moderate during phase III (Table 1). It has to be therefore assumed that carbon missing from the budget is largely dissolved organic carbon shown as DOC<sub>calc</sub>. Only for phase III DOC<sub>calc</sub> should be interpreted with caution. On the last sampling day (t27) undetermined pools (mainly wall growth) might have been contributing up to 5.5 of the 19 µmol kg<sup>-1</sup> DOC<sub>calc</sub>. Visual inspections by divers indicated that large parts of wall growth were removed by the brushing technique, but measurements most likely underestimated wall growth.

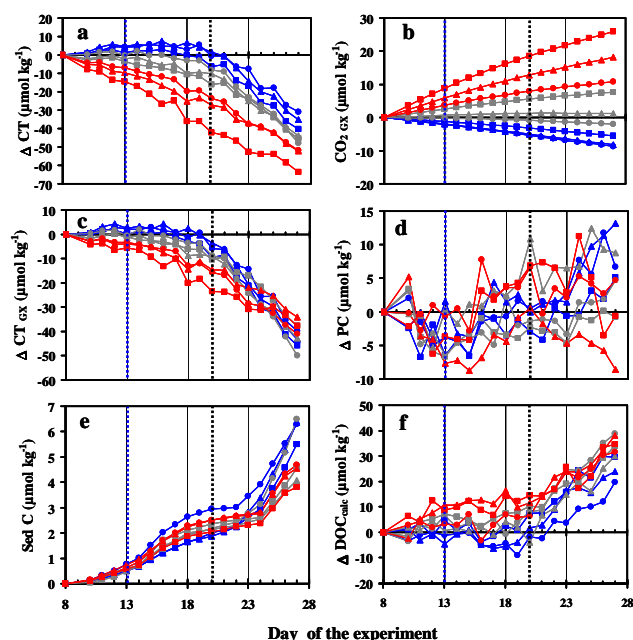


**Fig. 4.** Temporal development of carbon pools in the control treatment. (a) shows daily measured differences in particulate carbon (ΔPC), total inorganic carbon (ΔCT), dissolved organic carbon calculated from mass balance (DOC<sub>calc</sub>), cumulative sedimented carbon (Sed) and CO<sub>2</sub> gas exchange (GX) relative to day zero for the lowest CO<sub>2</sub> treatment (175 µatm). In (b), measured changes in dissolved organic carbon (ΔDOC) and Pool X (the carbon fraction that cannot be accounted for by changes in combined daily measured pools) are included. (c) compares DOC<sub>calc</sub> of the 175 µatm treatment with DOC<sub>calc</sub> of the second control mesocosm (180 µatm). The black dashed line denotes the end of phase II comprising the second bloom peak; phase III corresponds to the third bloom until the end of measurements.

### 3.3 CO<sub>2</sub> effects on carbon budgets

Changes in inorganic carbon concentrations between t8 and t27 (Fig. 5a) are correlated to CO<sub>2</sub> levels with the highest CO<sub>2</sub> treatment showing the strongest decrease in CT. At high *p*CO<sub>2</sub> the decrease was rather linear over time, whereas there was only a minor change in CT concentrations in the low CO<sub>2</sub> treatments during the first 12 days of this period. Most of these CO<sub>2</sub>-related differences were caused by gas exchange with the atmosphere. One third (24 µmol kg<sup>-1</sup>) of the CT decrease at the highest CO<sub>2</sub> over the whole





**Fig. 5.** Temporal development of carbon pools in all treatments relative to day 8. Changes in dissolved inorganic carbon ( $\Delta\text{CT}$ ) (a) corrected for gas exchange  $\text{CO}_2\text{_{GX}}$  (b) can be used to calculate biologically mediated changes in inorganic carbon concentrations ( $\Delta\text{CT}_{\text{GX}}$ ) (c). This production estimate together with changes in particulate carbon ( $\Delta\text{PC}$ ) (d) and cumulative sedimentation of particulate carbon (Sed) (e) were used to estimate dissolved organic carbon from mass balance  $\Delta\text{DOC}_{\text{calc}}$  (f).  $\text{CO}_2$  treatment levels and vertical dashed lines are coded as described for Fig. 3.

experiment can be attributed to outgassing, whereas about 25 % ( $10\text{ }\mu\text{mol kg}^{-1}$ ) of the carbon consumed in the lowest  $\text{CO}_2$  treatments entered from the atmosphere (Fig. 5b). The gas exchange corrected variation of  $\Delta\text{CT}$  (Fig. 5c),  $\Delta\text{CT}_{\text{GX}}$  is an approximation for biological net inorganic carbon fixation, as calcification was negligible during the experiment (Silyakova et al., 2012). Approximately equal amounts of inorganic carbon were consumed biologically in all treatments between t8 and t27. However, uptake rates often strongly differed among  $\text{CO}_2$  treatments. CT was taken up immediately and rather continuously at high  $\text{CO}_2$ . At low  $\text{CO}_2$ , there was a considerable lag phase until t20, but then net uptake rates exceeded rates at high  $\text{CO}_2$ , so that all treatments ended up with similar uptake at the end of phase III. Overall,  $\Delta\text{PC}$  build-up calculated from t8, with maximal  $10\text{ }\mu\text{mol kg}^{-1}$  (Fig. 5d), was rather small compared to net carbon uptake  $\Delta\text{CT}_{\text{GX}}$  of  $\sim 40\text{ }\mu\text{mol kg}^{-1}$ . Cumulative sedimentation can only account for half as much as  $\Delta\text{PC}$  build-up (up to  $5\text{ }\mu\text{mol kg}^{-1}$ , Fig. 5e). As the build-up of both measured particulate carbon species ( $\Delta\text{PC}$  and sediment) cannot explain the measured drawdown in  $\Delta\text{CT}_{\text{GX}}$ ,  $\Delta\text{DOC}_{\text{calc}}$  is accumulating over time (Fig. 5f).

### 3.4 Phase I

By separating the budget into three phases of the experiment, changes in elemental pools in the nine mesocosms can be directly compared using regression analyses.

Because inorganic nutrients were low at the beginning of phase I, there was no apparent uptake and inorganic nutrient budgets were thus not calculated. However,  $\Delta\text{CT}_{\text{GX}}$  (Fig. 6a) showed a relatively strong decrease of inorganic carbon at high  $\text{CO}_2$  compared to net heterotrophic production at low  $\text{CO}_2$ . This highly significant  $\text{CO}_2$  effect on carbon uptake was not reflected in particulate carbon accumulation (Fig. 6b, Table 3).  $\Delta\text{PC}$  was decreasing from the t8 bloom peak, while sedimentation was equally low in all treatments. This translates into a significant positive correlation of  $\Delta\text{DOC}_{\text{calc}}$  to  $\text{CO}_2$  in phase I (Table 3), causing an offset towards elevated  $\Delta\text{DOC}_{\text{calc}}$  at high  $\text{CO}_2$  that persisted over most of the experimental time (Fig. 5f).

### 3.5 Phase II

During phase II, dissolved inorganic matter (DIM) concentrations, comprising inorganic carbon (Fig. 6e) together with inorganic nutrients, decreased significantly stronger at high  $\text{CO}_2$  (Table 3). Although statistical tests could not detect a significant  $\text{CO}_2$ -related increase in all pools of dissolved and particulate organic matter, significant positive trends in Chl *a* (Fig. 3),  $\Delta\text{PON}$ ,  $\Delta\text{DOP}$  and  $\Delta\text{DOC}_{\text{calc}}$  (Fig. 6h, Table 3) suggest a general positive effect of  $\text{CO}_2$  on phytoplankton production during phase II. Mass balance indicates that surplus carbon taken up at high  $\text{CO}_2$  accumulated in the dissolved organic pool ( $\Delta\text{DOC}_{\text{calc}}$ ), while nitrogen remained mainly in the particulate fraction and phosphorus was almost equally partitioned between both the particulate and dissolved organic pool (Table 3). The small sedimentation event peaking on t16 can be mainly allocated to Cirripedia larvae migrating into the sediment trap (Figs. 7a, 6g). The settling of these meroplanktonic larvae decreased metazooplankton biomass by 40–60 % (from overall average  $5.0 \pm 1.8$  to  $3.1 \pm 1.1\text{ }\mu\text{mol kg}^{-1}$ ) over the course of the experiment, thereby contributing  $\sim 60\%$  to the export flux during phases I and II (see also Niehoff et al., 2013). A sedimentation event caused by sticky nets of pteropods introduced into the mesocosm during phase I was not detected. The fairly large dead individuals were excluded from the sediment samples.

### 3.6 Phase III

In phase III,  $\text{CO}_2$ -related trends in dissolved inorganic matter uptake rates of phase II are reversed. Significantly more inorganic carbon was consumed at low  $\text{CO}_2$  (Fig. 6i), resulting in significantly stronger build-up of PC (Fig. 6j) and export of this material (Fig. 6k). Trends in inorganic carbon uptake during phase III were parallel to trends in uptake of

**Table 3.** Results of *F* tests (regression analyses) for CO<sub>2</sub> dependent changes in pool sizes of C, N and P during the three phases of the experiment. Analysed delta values for each phase are calculated using the first day of the respective phase as a reference point. Mean delta concentrations of the elements in particulate organic (POM), dissolved inorganic (DIM) and dissolved organic matter (DOM) as well as sediments (Sed.) and pools calculated by mass balance (DOC<sub>calc</sub> DON<sub>calc</sub> POP<sub>calc</sub>) were plotted against mean CO<sub>2</sub> concentrations during the phase. Significant trends (*p* < 0.05) are marked grey. Direction (Dir) of trends are given for correlations *p* < 0.1. “Neg.” stands for negative correlation to *p*CO<sub>2</sub> and would for example mark a significantly stronger decrease in dissolved inorganic matter (DIM) with increasing *p*CO<sub>2</sub>, while “Pos.” stands for positive correlation to *p*CO<sub>2</sub> and could therefore mark significantly increasing concentrations, for example, POM with increasing *p*CO<sub>2</sub>.

Testet period		ΔPOM			ΔDIM			ΔDOM			Sed.			ΔOM <sub>calc</sub>		
		<i>r</i> <sup>2</sup>	<i>p</i>	Dir	<i>r</i> <sup>2</sup>	<i>p</i>	Dir	<i>r</i> <sup>2</sup>	<i>p</i>	Dir	<i>r</i> <sup>2</sup>	<i>p</i>	Dir	<i>r</i> <sup>2</sup>	<i>p</i>	Dir
Phase I	Carbon T8–T15	0.01	0.78	–	0.98	< 0.00	Neg.	0.42	0.06	Pos.	0.02	0.75	–	DOC <sub>calc</sub> 0.96	< 0.00	Pos.
Phase II	Carbon T14–T21	0.27	0.15	–	0.92	< 0.00	Neg.	0.01	0.77	–	0.00	0.92	–	DOC <sub>calc</sub> 0.66	0.01	Pos.
	Nitrogen T14–T21	0.52	0.03	Pos.	0.87	< 0.00	Neg.	0.25	0.17	–	0.02	0.69	–	DON <sub>calc</sub> 0.07	0.50	–
	Phosphorus T14–T21	0.35	0.10	–	0.93	< 0.00	Neg.	0.54	0.02	Pos.	0.02	0.71	–	POP <sub>calc</sub> 0.13	0.34	–
Phase III	Carbon T21–T27	0.86	< 0.00	Neg.	0.85	< 0.00	Pos.	0.07	0.49	–	0.65	0.01	Neg.	DOC <sub>calc</sub> 0.35	0.09	Neg.
	Nitrogen T21–T30	0.83	< 0.00	Neg.	0.89	< 0.00	Pos.	0.13	0.35	–	0.63	0.01	Neg.	DON <sub>calc</sub> 0.25	0.17	–
	Phosphorus T21–T30	0.31	0.12	–	0.94	< 0.00	Pos.	0.15	0.31	–	0.64	0.01	Neg.	POP <sub>calc</sub> 0.47	0.04	Neg.

inorganic nutrients (Table 3). As inorganic N and P were supplied in the beginning of phase II in equal concentrations (5 μmol kg<sup>−1</sup> DIN, 0.32 μmol kg<sup>−1</sup> DIP), stronger uptake at high CO<sub>2</sub> during phase II resulted in lowered concentrations of the limiting nutrients during phase III. On t20, concentrations of ~3.5 μmol kg<sup>−1</sup> DIN and ~0.2 μmol kg<sup>−1</sup> DIP were available in the control treatments, but only approximately half of this amount in the highest CO<sub>2</sub> treatment (1.76 and 0.10 μmol kg<sup>−1</sup> DIN and DIP, respectively). DIN and DIP were depleted in all mesocosms at about day t28. Uptake rates were considerably faster in the still relatively nutrient-replete low CO<sub>2</sub> treatments. On the last days of the experiment, net community production at low CO<sub>2</sub> exceeded all rates observed before during this experiment. CO<sub>2</sub>-related trends in ΔPC, ΔPON and especially in export of C, N and P could be clearly detected. Changes in nearly all of the particulate pools showed highly significant negative correlations to treatment *p*CO<sub>2</sub> (Table 3).

DOC<sub>calc</sub> (Fig. 6l) was steadily increasing until day 27 in all CO<sub>2</sub> treatments. Major parts of the nutrients added on t13 accumulated in those pools determined by mass balance until the end of the experiment on t30 (averaged ~55 % in DON<sub>calc</sub> and 74 % in POP<sub>calc</sub>). Whereas 16 % of added N and 32 % of added P was measured as wall growth on t30 on average over all mesocosms. A CO<sub>2</sub> effect on wall growth is neither indicated by direct measurements on t30 nor by CO<sub>2</sub> correlations of DOC<sub>calc</sub>, DON<sub>calc</sub> or POP<sub>calc</sub>, to which it was contributing during phase III.

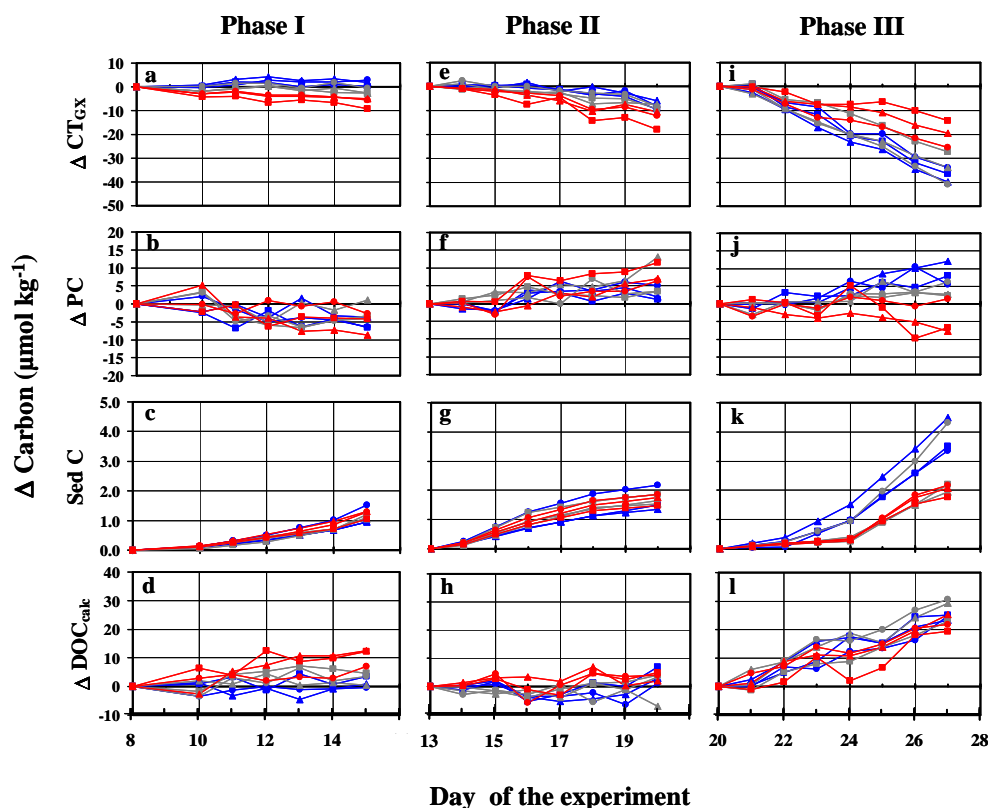
### 3.7 Stoichiometry of particulate matter

Elemental ratios of water-column particulate matter were always close to Redfield ratios (Fig. 8). CO<sub>2</sub> or nutrient manipulation did not cause strong changes in elemental composition of particulate matter. C/N and N/P ratios of material sampled from the sediment traps were generally similar to ratios obtained for water-column particulate matter, with mean N/P ratios of the sediment slightly higher than respective values from the water column. During the last days (t27–t30) of the experiment, C/N ratios of water-column particulate matter increased in all mesocosms (Fig. 8a). This increase was significantly stronger for the low CO<sub>2</sub> treatments. The same general increase was observed in the sediment, but here C/N started to rise already on t24, reaching higher maximum values than in the water column (Fig. 8b). Diatom aggregates contributed the largest fraction of the sediment during the last week of the experiment as documented by a strong increase in biogenic silica fluxes (Fig. 7b), an elevated Si/C ratio (Fig. 7c) and microscopic inspection.

## 4 Discussion

### 4.1 Phase I

In the control treatments, dynamics in total inorganic carbon and gas exchange over the first 19 days of the experiment are well represented by measured production, export and respiration of particulate organic matter (Fig. 4a). Thus,

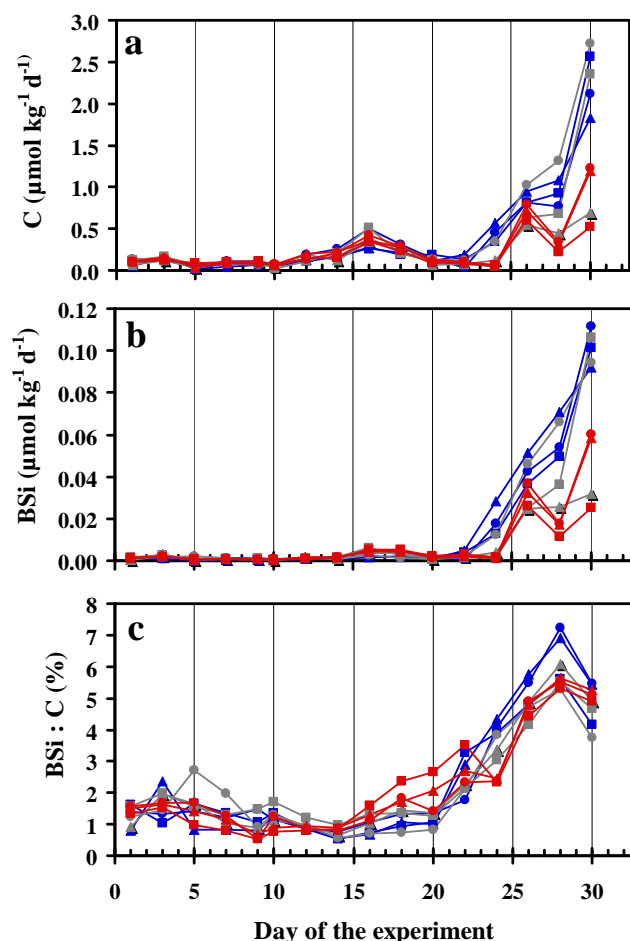


**Fig. 6.** Temporal development of carbon pools in all treatments for the three phases of the experiment. Biologically mediated changes in inorganic carbon concentrations ( $\Delta\text{CT}_{\text{GX}}$ ) together with changes in particulate carbon ( $\Delta\text{PC}$ ) and cumulative sedimentation of particulate carbon during each phase (Sed) were used to estimate dissolved organic carbon from mass balance  $\Delta\text{DOC}_{\text{calc}}$ . CO<sub>2</sub> treatment levels and vertical dashed lines are coded as described for Fig. 3.

the carbon budget was closed with  $\text{DOC}_{\text{calc}}$  approaching zero at low CO<sub>2</sub>. At high CO<sub>2</sub>, however, there was significantly higher carbon consumption during phase I (Figs. 5c, 6a). Due to a strong vertical light gradient, <sup>14</sup>C gross primary production measured at 1 m water depth was high, showing a clear, positive correlation to CO<sub>2</sub> (Engel et al., 2013). In contrast, oxygen based production estimates by Tanaka et al. (2013) incubated at 3 m water depth were low and seem to be already partly balanced by respiration. The extra carbon ( $\sim 13 \mu\text{mol kg}^{-1}$ ) consumed at high CO<sub>2</sub> was not found in any of the particulate carbon pools (Fig. 6b, c), it was thus allocated to  $\text{DOC}_{\text{calc}}$ . Despite strong variability, statistical analyses of DOC measurements as well as <sup>14</sup>C DOC primary production measurements by Engel et al. (2013) support our interpretation of increased DOC production at high CO<sub>2</sub> in the phase before nutrient addition. DOC production was probably related to phytoplankton exudation but also to viral lyses of nanoplankton and microzooplankton grazing, both initiating the decline of the phase I bloom (Brussaard et al., 2013). Stronger microzooplankton grazing at low CO<sub>2</sub> during phase I was stated by de Kluijver et al. (2013) and Brussaard et al. (2013). Enhanced primary production at high CO<sub>2</sub> (Engel et al., 2013) presumably in combination with op-

posing strong heterotrophic loss processes at low CO<sub>2</sub> (Brussaard et al., 2013) were resulting in net uptake of inorganic carbon in the future CO<sub>2</sub> treatments and concomitant release of inorganic carbon in the low CO<sub>2</sub> controls (Fig. 6a). Rapid cycling of carbon during phase I obviously resulted in accumulation of DOC at high CO<sub>2</sub> that was not readily bioavailable and persisted at least during phase II, in which net production of DOC was considerably lower (Fig. 5f, see also Engel et al., 2013). A semi-labile nature of the produced dissolved organic matter in this experiment becomes also evident from apparent substrate limitation of heterotrophic bacteria during the entire experiment, detected by Piontek et al. (2013).

The phenomenon of inorganic carbon drawdown with low net consumption of N or P, called carbon over-consumption (Toggweiler, 1993), was enhanced at high CO<sub>2</sub> between t8 and t15. Such feature was already found in incubations of natural Atlantic plankton communities under high CO<sub>2</sub> (Schulz et al., 2008; Bellerby et al., 2008; Riebesell et al., 2007; Delille et al., 2005; Engel et al., 2004a; Egge et al., 2009; Hein and Sand-Jensen, 1997) and is also indicated by increased cumulative <sup>14</sup>C primary production observed during the experiment by Engel et al. (2013). The final product



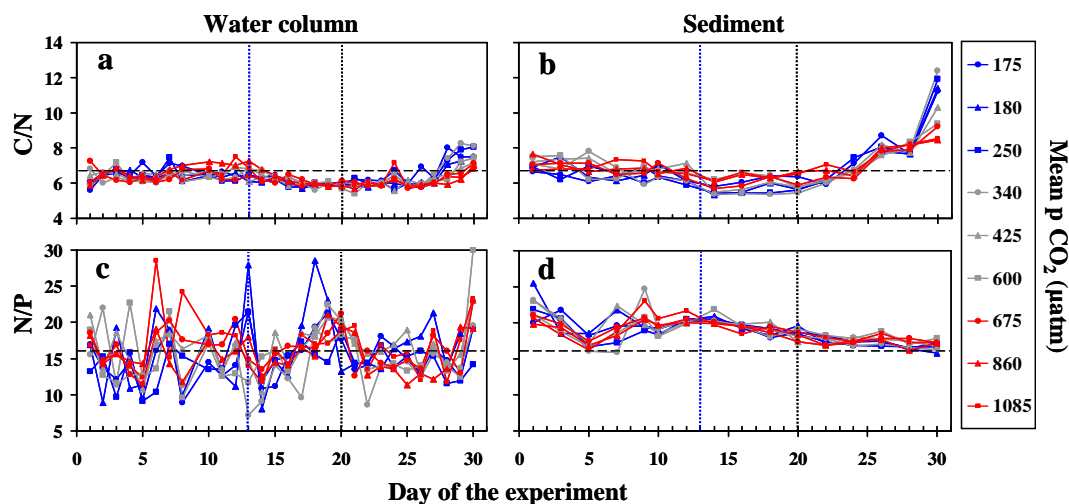
**Fig. 7.** Vertical fluxes for carbon (a) and biogenic silica (b) normalised to kg seawater and day. Biogenic silica to carbon ratios in the sedimented material are shown in (c). Treatment levels and vertical dashed lines are coded as described for Fig. 3.

of enhanced photosynthetic activity could often not directly be measured, but was hypothesised to be released as DOC, subsequently occurring as TEP (Engel et al., 2004a). Sedimentation of aggregated DOC in the form of TEP was hypothesised to cause increased carbon loss under high CO<sub>2</sub> in the PEECE (Pelagic Ecosystem CO<sub>2</sub> Enrichment) III study (Schulz et al., 2008) and in further previous experiments (Engel et al., 2004b). In this experiment, an increased contribution of freshly fixed <sup>13</sup>C to high CO<sub>2</sub> sediments during phase I was argued to be caused by sinking of TEP by de Kluijver et al. (2013). TEP formation implies a flux of DOC into the PC pool, but neither increased bulk sedimentation nor increased carbon contents of sediments or of water-column particulate matter under high CO<sub>2</sub> were measured. Sediment fluxes were low and indication for TEP as a relevant aggregation factor was not detected. Sugars serving as precursors for TEP were possibly produced at concentrations too low to form relevant amounts of aggregates or were consumed by the bacterial community right after release (Pio-

ntek et al., 2010). DOC was therefore remaining in the water column, where it, on the long run, might support the development of the microbial community. Although an increase in bacterial numbers was not observed during the duration of the experiment (Brussaard et al., 2013), DOC accumulation rather promotes enhanced shallow remineralisation than primary production or export (Thingstad et al., 2008).

## 4.2 Phase II

During phase II the trends in uptake of inorganic C, N and P after nutrient addition were consistent with trends in Chl *a*. Elevated CO<sub>2</sub> concentrations seemed to have a stimulating effect on growth of the dominating picoeukaryotic primary producers (Brussaard et al., 2013; Schulz et al., 2013), leading to increased uptake of inorganic matter (Table 3, Fig. 6e), as well as increased cell numbers and Chl *a*. However, increasing biomass production as a result of enhanced nutrient uptake at high CO<sub>2</sub> during the bloom of picoeukaryotes in phase II was hardly detectable as particulate organic matter build-up and did not cause a significant sedimentation event (Figs. 6f, 5g). A peak in carbon export caused by Cirripedia larvae (t16, Fig. 7a) shows that the settling of meroplankton can seasonally account for a large portion of vertical fluxes in coastal ecosystems. This finding is in agreement with <sup>13</sup>C tracer data by de Kluijver et al. (2013). Generally low enrichment of sediment with the tracer during phases I and II can only be explained by high contents of zooplankton and “old” detrital material. Build-up of autotrophic biomass in the water column, estimated from Chl *a* as well as from biovolume of picoeukaryotes (Brussaard et al., 2013) can only account for the amount of inorganic C, N and P consumed during the first days of phase II. Thereafter, phytoplankton standing stocks were diminished and kept at a relatively low level by microzooplankton grazing and viral lyses of picoeukaryotes (Brussaard et al., 2013). Nutrient and carbon uptake continued during the time of strong biomass loss. Low C/N and C/P inorganic uptake ratios in all treatments suggest overall elevated production of organic P and N compared to organic C during phase II (Silyakova et al., 2012). Significant CO<sub>2</sub>-correlated differences in ΔPC as well as ΔPOP<sub>calc</sub> could not be detected during the entire phase II (Table 3). Dissolved organic matter is indeed showing positively CO<sub>2</sub>-related differences in ΔDOC<sub>calc</sub> (Fig. 6h) but also in ΔDOP. Release of cell contents rather than exudation may have been the source of this DOM at nutrient-replete conditions, as enhanced autotrophy at high CO<sub>2</sub> was strongly top-down controlled (Brussaard et al., 2013). In contrast to P and C, enhanced uptake of inorganic nitrogen at high CO<sub>2</sub> was also reflected in higher ΔPON and only minor amounts of N accumulated in DON<sub>calc</sub>. Nitrogen was obviously preferentially incorporated into the heterotrophic community compared to C and P (Table 3).



**Fig. 8.** Temporal development of measured carbon to nitrogen (C/N) and nitrogen to phosphorus (N/P) ratios of particles suspended in the water column (left) and particles sampled in the sediment traps (right). Dashed horizontal line marks Redfield ratios. CO<sub>2</sub> partial pressures given in the figure legend are mean values for the experiment (day 8–26). Vertical dashed lines are coded as described for Fig. 3.

### 4.3 Phase III

A relatively diverse phytoplankton community comprised of picoeukaryotes, co-dominated by diatoms and dinoflagellates established during phase III (Schulz et al., 2013; Brussaard et al., 2013). Biomass build-up resulting from fast carbon and nutrient drawdown did not seem to be limited by strong heterotrophic loss processes as observed in the previous two phases. This bloom showed higher C, N and P uptake rates at low CO<sub>2</sub>, whereas under high CO<sub>2</sub> uptake was already slowing down due to nutrient depletion after the phase II peak (Fig. 6i). Reduced growth at high CO<sub>2</sub> in phase III was reflected by significantly lower  $\Delta PC$ ,  $\Delta PON$ , and  $\Delta POP$  (Table 3). Diatoms, contributing relatively low biomass to the pelagic community (Schulz et al., 2013), increasingly dominated the sediment material from the beginning of phase III in all treatments. In the sediment material, this is illustrated by increasing BSi/C ratios from t20 onwards (Fig. 7c), concurring with microscopic observations of large quantities of diatom chains and their fatty acid signature detected by de Kluijver et al. (2013). Overall, higher biomass build-up seemed to be causing a sedimentation event of diatoms, forming aggregates with other particles, transporting them into the sediment trap. In contrast to the previous two phases, <sup>13</sup>C tracer concentrations indicate that there were even higher contributions of freshly fixed carbon to sediments than to water-column PC (de Kluijver et al., 2013). The retention food web, quickly recycling freshly produced biomass, seems to have shifted towards an export community in phase III (Wassmann, 1997). Strongly reduced export at high CO<sub>2</sub> was obviously due to nutrients being diminished by enhanced growth during phase II. Unfortunately, the total amount of export production cannot be calculated for this period, as measurements ended before peak sedimentation

rates were reached (Fig. 7a). In addition to that, DOC<sub>calc</sub> and wall-grown biomass, not being exported, were exponentially increasing (Fig. 4c). However, an export flux at high CO<sub>2</sub> comparable to fluxes observed at low CO<sub>2</sub> occurring after the end of our measurements (t30) seems unlikely, as inorganic N and P were depleted at t28. Until nutrient depletion, significantly less carbon uptake, PC and PON accumulation in the water column and less export of C, N and P were observed at high CO<sub>2</sub> during phase III.

### 4.4 Stoichiometry of exported particulate matter

Temporal trends in elemental composition of the sedimented particulate matter were very similar to that of the water column during most parts of the experiment (Fig. 8). Carbon isotope tracer data in sediments suggests that there was mostly an under-representation of phytoplankton biomass (de Kluijver et al., 2013). Elemental composition of sediment was overall similar to water column particulate matter. This classifies sinking material sampled at the bottom of the shallow mesocosms to be relatively fresh. Nevertheless, sinking detritus and digested material produced by the large heterotrophic community was probably responsible for the slight general offset in composition of sedimenting material towards higher C/N and N/P ratios throughout the experiment. While changes in particulate matter composition in response to future CO<sub>2</sub>, reported from laboratory experiments on phytoplankton (Burkhardt et al., 1999; Burkhardt and Riebesell, 1997), are varying between species in strength and direction, differences in organic C/N/P ratios of primary producers growing under inorganic nutrient limitation and depletion are a common phenomenon (Klausmeier et al., 2004). The fact that we did not observe strong changes in response to nutrient or CO<sub>2</sub> addition, during this experiment is likely a



result of the diverse composition of POM, with phytoplankton comprising only a relatively small fraction during large parts of the experiment. Fast recycling of readily available N and P compounds during phase I were obviously covering the nutrient demand of phytoplankton (Schulz et al., 2013) and probably comprised a large part of the fluxes even during nutrient replete phases of the experiment.

The observed increase of C/N ratios in the sediment compared to the water-column particulate matter during phase III correlates with the occurrence of diatoms and increasing silicate flux (Fig. 7c). This diatom ballast or aggregation effect caused the over-representation of fresh carbon-rich particles in the sediments. The same C/N increase as in the sediment occurred later and less pronounced in the water column; here diatoms and fresh material are measured against a much larger background of other water-column particulate matter, thus diluting this signal.

On t30, higher C/N ratios at low CO<sub>2</sub> appeared in water-column particulate matter. Similar to the general increase of C/N in all mesocosms, also the CO<sub>2</sub> effect on C/N was amplified in the sediment. Mesocosms showing stronger elevated C/N ratios on the last day had also higher sedimentation rates of C, N, P and Si (Table 3). Carbon uptake beyond the day of nutrient depletion could not be determined, as the inorganic carbon dataset ends on t27. But already until t27 inorganic carbon to nutrient uptake was much higher at low CO<sub>2</sub> during phase III and thus even during the combined post nutrient phase (II + III) (Silyakova et al., 2012). A combination of high uptake rates and the shift towards increased C/N on the last days overcompensated initially higher uptake at high CO<sub>2</sub> during phase II. Elevated C or reduced N content of the phytoplankton community might have been due to growth limitation by dissolved inorganic nitrogen with concentrations below 1  $\mu\text{mol kg}^{-1}$  (Klausmeier et al., 2004) already since t24 in all treatments.

#### 4.5 Synthesis

Despite all efforts in determining all relevant elemental pools of C, N and P in regular temporal intervals, budgets could not readily be closed. This was mainly because of unsatisfying precision in DOC, DON and POP as well as wall growth towards the end of the experiment. However, gaps in the budget could be narrowed down by introducing novel methodology to determine gas exchange and sedimentation. It could be demonstrated that measured pools in large mesocosms can be quantitatively evaluated against each other. Moreover, using mass balance, problematic measurement parameters as well as technical and operational problems could be identified and their relevance could be quantified. In later experiments, improved sediment traps, an improved DOC sampling strategy as well as regular cleaning of the walls solved the most prominent problems pointed out in this study (see improved methods in Riebesell et al., 2013). The particular rea-

son for the lack in precision in DON and POP within this dataset could not be ascertained.

Future CO<sub>2</sub> concentrations were found to stimulate autotrophic production twice during the course of this experiment. First during phase I, when increased inorganic carbon uptake by nanoplankton at high CO<sub>2</sub> was directly channelled into dissolved organics. Secondly, during phase II, when enhanced growth of picoeukaryotes diminished inorganic nutrient concentrations at high CO<sub>2</sub>, resulting in less organic matter being exported in phase III. During this experiment, both positive effects on primary producers had negative influence on carbon export.

After phase I, community composition and carbon flows had changed. Later effects were therefore a product of complex resource competition and cascading loss processes modified by CO<sub>2</sub> and preceding production. The dominating producers or consumers in a mesocosm had responded to the manipulation at the beginning of the experiment, any following effects were multi-causal. Growth enhancement of nanoplankton and picoeukaryotes during phase I and II (Brussaard et al., 2013; Engel et al., 2013; de Kluiver et al., 2013) might have caused most of the CO<sub>2</sub> effects on bloom dynamics observed during and after their occurrence. Therefore, e.g. direct physiological CO<sub>2</sub> effects on plankton dominating within phase III would be a matter of speculation due to the different nutrient situation already in the beginning of phase III. This demonstrates that ecological data are of substantial importance in making biogeochemical response patterns comparable between experiments. Apparently, responses found for the retention type community, which was present at the start of this experiment, are not directly comparable to earlier findings for export systems such as the coccolithophore blooms in Norwegian coastal waters compiled by Riebesell et al. (2008). Community experiments always have to be seen as case studies with results primarily valid for the specific community composition enclosed at the start of the experiment. Further experiments will show whether CO<sub>2</sub> enhanced DOC production and growth of smaller phytoplankton can be consistently found at similar community composition.

Laboratory studies performed on single species are ideal to detect physiological CO<sub>2</sub> effects, whereas the importance of these effects within a natural ecosystem is always difficult to extrapolate (Riebesell and Tortell, 2011). In this experiment, ocean acidification/carbonation affected small phytoplankton, which in turn significantly influenced principle ecosystem functioning. Whereas enhanced growth of picoeukaryotes itself had no immediate effect on carbon export fluxes, their footprint in the nutrient budget controlled export fluxes later during the experiment. Identifying species that have the potential to change biogeochemical fluxes by influencing community succession is an important task for future mesocosm experiments. Focussing from the community to the species level instead of extrapolating from the laboratory



to the field could accelerate the progress of finding general biogeochemical response patterns.

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